Pharmacokinetics and Metabolism of Benzene in Zymbal Gland and Other Key Target Tissues after Oral Administration in Rats

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Solid tumors have been reported in the Zymbal gland, oral and nasal cavities, and mammary gland of Sprague-Dawley rats following chronic oral administration of benzene. The cause for the specificity of such lesions remains unclear, but it is possible that tissue-specific metabolism or pharmacokinetics of benzene is responsible. Metabolism and pharmacokinetic studies were carried out in our laboratory with ^{14}C-benzene at oral doses of 0.15 to 500 mg/kg to ascertain tissue retention, metabolite profile, and elimination kinetics in target and nontarget organs and in blood. Findings from thoses studies indicate the following: a) the Zymbal gland is not a sink or a site of accumulation for benzene or its metabolites even after a single high dose (500 mg/kg) or after repeated oral administration; b) the metabolite profile is quantitatively different in target tissues (e.g., Zymbal gland, nasal cavity), nontarget tissues and blood; and c) pharmacokinetic studies show that the elimination of radioactivity from the Zymbal gland is biphasic.

Introduction

Two-year bioassay studies by Maltoni et al. (1,2) and by the National Toxicology Program (NTP) (3) have demonstrated that chronic oral administration of benzene in rats produces solid tumors in a number of organs, including Zymbal gland, nasal and oral cavities, and mammary gland. It is generally believed that the toxic effects of benzene result from the metabolism of benzene to electrophilic intermediates capable of interacting covalently with critical macromolecules (4–8). Although the exact chemical nature of the ultimate toxic or carcinogenic species remains unknown, several metabolic pathways proposed for benzene lead to the formation of reactive intermediates. Of importance are those pathways giving rise to mono- and polyhydroxylated metabolites (e.g., phenol, hydroquinone, catechol, 1,2,4-benzenetriol), ring-opened metabolites (e.g., muconaldehyde, muconic acid) and biphenolic metabolites (e.g., 4,4'-biphenol) (9–15). The metabolism of benzene has been extensively studied in the liver and bone marrow (4,19–21), but little effort has been directed toward investigating the metabolism of benzene in other tissues (22,23). Target organ susceptibility to the carcinogenic or toxic effects of xenobiotics is, however, thought to be governed by many factors with tissue-specific metabolism being of key importance (24,25); such metabolism might lead to the formation and persistence, at critical levels, of genotoxic metabolites.

The studies reported in this paper were designed to investigate the metabolism and pharmacokinetics of benzene in the Zymbal gland and other solid tumor target organs in Sprague-Dawley rats after oral administration. Results were evaluated to delineate differences in the metabolite profile, elimination kinetics, and bioaccumulation of benzene and/or its metabolites in Zymbal gland, nasal and oral cavities, and mammary gland, which are considered here to be target tissues, and liver and kidney, which are considered to be nontarget tissues.

Materials and Methods

Materials

[UL-^{14}C]Benzene (80–100 mCi/mmole) was purchased from Chemsyn Science Laboratories (Lenexa, KS); radiochemical purity of this material was determined by RP-HPLC analysis (70% MeOH-30% water, v/v) to be greater

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than 99%. Benzene was obtained from American Burdick and Jackson (Muskegon, MI) (99.7% pure by GC analysis). Metabolite standards (phenol, hydroquinone, catechol, 1,2,4-benzenetriol, 2,2'-biphenol, 4,4'-biphenol, trans,trans-muconic acid, phenyl-β-D-glucuronide) were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO) and were greater than 99% pure. Phenylsulfate was isolated as a metabolite from the urine of rats treated with ^{14}C-benzene, characterized by selective enzymatic hydrolysis with sulfatase, and purified by HPLC. Glucuronide conjugates of hydroquinone, catechol, and 1,2,4-benzenetriol were synthesized enzymatically using uridine diphosphate [^{14}C]glucuronic acid (UDPGA) (304 mCi/mole, New England Nuclear) or unlabeled UDPGA and rat liver microsomes (22) and purified by HPLC. Tetrabutylammonium dihydrogen phosphate, ascorbic acid, and ammonium acetate were obtained from Aldrich Chemical Co. and olive oil from Sigma Chemical Co. HPLC grade solvents (acetonitrile, methanol and tetrahydrofuran) were purchased from J. T. Baker (Phillipsburg, NJ).

Animals

Female Sprague-Dawley [Crl:CD(SD)BR] rats were obtained from Charles River Laboratory (Kingston, NY). Rooms were maintained at 20 to 22°C with a relative humidity of 40 to 60% and a 12-hr light/dark cycle. Animals received food (rodent chow, #5002 pellets, Ralston Purina, St. Louis, MO) and tap water (automatic water feeder) ad libitum. At study initiation, rats were 12 to 18 weeks of age and weighed 225 to 375 g.

Methods

Oral Gavage Studies and Tissue Collection. Sprague-Dawley rats received single doses by oral gavage of ^{14}C-benzene, at 0.15, 1.5, 15, 150, and 500 mg/kg, in olive oil (~ 0.8–1.0 mL per animal). The specific activity of the oral gavage doses was adjusted such that each animal received 30 to 60 μCi. Generally, groups of three animals were sacrificed at 1, 3, 6, 9, 12, and 24 hr after oral administration and blood (cardiac puncture), Zymbal gland, nasal and oral cavity tissues, mammary gland tissue, bone marrow (femur), liver, and kidney were collected. Samples were frozen immediately after necropsy and stored at −70°C until analyzed. One experiment was carried out in which three rats received ^{14}C-benzene for 2 weeks (500 mg/kg daily, 5 days a week for 2 weeks) and were sacrificed on day 14 one hr after the last radioactive dose. The amount of radioactivity in the Zymbal gland was determined.

Radioactivity Analyses. Determination of total radioactivity, in target and nontarget tissues, and blood was carried out by measuring the amount of ^{14}CO$_2$ produced from combustion of the samples or a homogenate of the samples. When homogenization of the sample was performed, one equivalent volume (mL) of cold water or cold 0.1 M ascorbic acid solution was added for each gram of wet tissue. Combustion was carried out for 3 min on a

Harvey Model OX-300 instrument. Radioactivity measurements were quantitated on a Beckman Model LS9000 liquid scintillation spectrometer using appropriate quench corrections. Tissue concentrations were calculated using specific activities of the radiolabeled benzene oral doses and are expressed in units of ^{14}C-benzene equivalents (ng or μg) per gram or per milliliter.

Metabolite Isolation and HPLC Analysis. Isolation of metabolites from blood and the tissues was carried out according to the following procedure: samples were homogenized or minced in cold 100 mM ascorbic acid solution [blood, 0.5 mL per 1 mL; liver, kidney, nasal and oral cavity tissues, mammary gland, 250–500 mg/ml; Zymbal gland (pooled), 50 mg/500 μL; bone marrow (pooled), 100–200 mg/500 μL]. Homogenates were extracted with two volumes of ethyl acetate to isolate the unconjugated metabolites, and ethyl acetate and aqueous layers were separated by centrifugation. The aqueous layer was extracted a second time with ethyl acetate and the organic fractions combined. Ethyl acetate was removed under a stream of nitrogen gas and the residue reconstituted in the HPLC mobile phase (5% acetonitrile-95% 0.1 M ammonium acetate, pH 4.0, v/v); separation of the unconjugated metabolites was carried out by HPLC using the conditions described below. The remaining aqueous fraction was treated with an equivolume of methanol to precipitate proteins, followed by centrifugation. The supernatant fraction was isolated and methanol removed under a stream of nitrogen gas; separation of the water-soluble, conjugated metabolites present in this aqueous sample was carried out by ion-pair HPLC as described below. HPLC analyses were performed on a Beckman model 330 binary gradient high performance liquid chromatographic system consisting of two model 110A pumps, a model 420 controller and a model 210 injector (Beckman Instruments, Fullerton, CA) or on a Varian 5020 binary gradient HPLC system with two single-piston solvent delivery pumps, an internal microprocessor controller and a Varian 9090 injector/autosampler (Varian Instruments, Walnut Creek, CA). Reverse-phase (RP) columns (Altex ODS, 4.6 mm × 15 cm or 25 cm) with Brownlee C8 (4.6 mm × 30 mm) guard columns were used in most of the HPLC analyses. Identification of radiolabeled benzene metabolites was based on comparison of retention times of radiolabeled HPLC peaks with those of standards. Detection of these standard metabolites was carried out by monitoring the UV absorbance (254 nm) of the HPLC effluent with a Beckman model 153 fixed wavelength instrument. Radiolabeled glucuronide and sulfate standards were monitored with a Berthold flow-through model LB506C radioactivity detector.

Radiometric-HPLC Analysis of Metabolites. Unconjugated metabolites present in the evaporated ethyl acetate sample were separated on an Altex ODS HPLC column (4.6 mm × 15 cm) using the chromatographic conditions reported by Lunte and Kissinger (26); elution was carried out with 5% acetonitrile-95% 0.1 M ammonium acetate (pH 4.0) (v/v) at a flow rate of 1 mL/min. The water-soluble metabolites were separated using an ion-

pair HPLC procedure slightly modified after the one reported by Sabourin et al. (22). Briefly, an Altex ODS column (4.6 mm × 25 cm) was initially equilibrated with 10% methanolic tetrabutylammonium dihydrogen phosphate (TBAP, 30 mM) / 90% aqueous TBAP (50 mM). Following injection, the HPLC column was eluted using a 10-min linear gradient from 10% methanolic TBAP to 45% methanolic TBAP at a flow rate 1 mL/min. At 10 min, the methanolic TBAP solvent was replaced with a solvent mixture containing 90% methanolic TBAP (30 mM) and 10% tetrahydrofuran (THF) (v/v) and the HPLC column eluted isocratically with 45% methanolic TBAP/THF (90:10) /55% aqueous TBAP. The column was allowed to reequilibriate with 10% methanolic TBAP /90% aqueous TBAP before another injection was made. The HPLC effluent was monitored with a Berthold Model LB506C flow-through radioactivity detector. Alternatively, fractions of the eluate (0.5 mL) were collected in scintillation vials and 15 mL of cocktail fluid (Ready Sovl CP, Beckman, Fullerton, CA) added to each vial. Radioactivity in the fractions was determined with a Beckman model LS9000 liquid scintillation instrument.

Results

Absorption and Distribution

The time course of disposition and elimination of radioactivity in Zymbal gland and other organs after oral administration of 0.15 and 1.5 mg/kg ^{14}C-benzene is shown in Figures 1 and 2. The highest levels of radioactivity for all tissues and organs were seen at the earliest sampling period (1 hr), suggesting that peak levels could have occurred somewhat earlier. However, levels differed among certain tissues and organs over the entire sampling period. One hour after single oral doses of 0.15 and 1.5 mg/kg ^{14}C-benzene (Table 1), concentrations of radioactivity fell roughly into three groups: the highest levels were found in liver and kidney; the lowest levels in Zymbal gland, nasal cavity tissue, oral cavity tissue, mammary gland, and bone marrow; and intermediate levels in blood. However, this distribution was altered at 15 mg/kg where disproportionate increases were found in mammary gland and bone marrow.
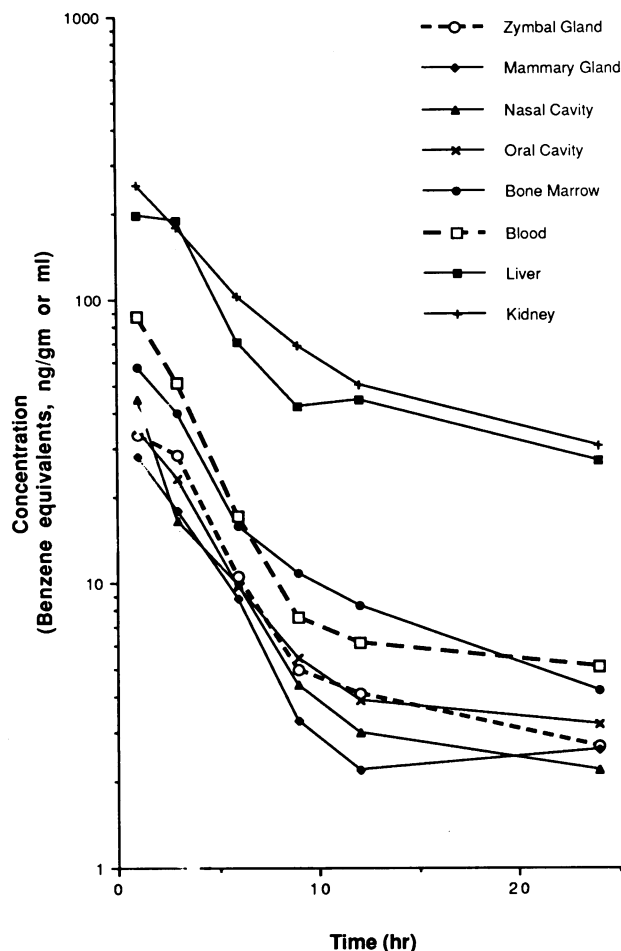

FIGURE 1. [14]C concentrations in the Zymbal gland, and various other target and nontarget organs in Sprague-Dawley rats following a single oral dose of benzene (0.15 mg/kg). Points represent the means of three animals.

FIGURE 2. [14]C concentration in the Zymbal gland and various other target and nontarget organs in Sprague-Dawley rats following a single oral dose of benzene (1.5 mg/kg). Points represent the means of three animals.
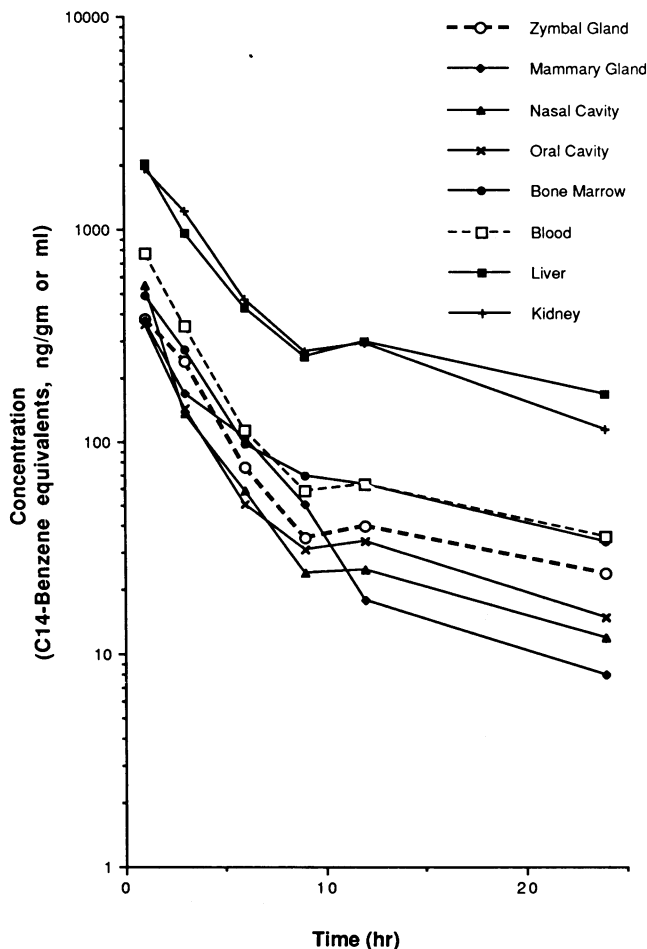
Table 1. Concentration of radioactivity in various tissues 1 hr following oral administration of 0.15, 1.5 and 15 mg/kg ^{14}C-benzene.

Tissue	μg Benzene equivalents/g or mL, ppm[a]		
	0.15 mg/kg	1.5 mg/kg	15 mg/kg
Zymbal gland	0.034 ± 0.006	0.380 ± 0.059	3.2 ± 0.4
Nasal cavity	0.044 ± 0.008	0.547 ± 0.123	2.4 ± 0.8
Oral cavity	0.035 ± 0.001	0.359 ± 0.017	2.4 ± 0.2
Mammary gland	0.028 ± 0.008	0.373 ± 0.055	6.6 ± 1.4
Blood	0.086 ± 0.004	0.769 ± 0.073	6.3 ± 0.9
Bone marrow	0.058 ± 0.005	0.490 ± 0.077	10.1 ± 1.3
Liver	0.198 ± 0.006	2.043 ± 0.195	12.8 ± 1.4
Kidney	0.254 ± 0.005	1.926 ± 0.174	12.2 ± 1.2

[a]Values represent mean ± SEM for three animals.

Elimination from Target Tissues

The elimination of radioactivity in the Zymbal gland occurred in a biphasic manner (Fig. 3); the ^{14}C elimination half-life for the rapid phase was 2.4 to 2.8 hr, while the half-life of the slow phase was 18 to 21 hr (Table 2). As evident in Figures 1 and 2, elimination of ^{14}C from the nasal and oral cavities, mammary gland, blood, liver, kidney, and bone marrow also displayed biphasic kinetics. During the first 8 to 10 hr, elimination of radioactivity occurred rapidly in these tissues; but after this period, radioactivity disappeared at a much slower rate. The half-lives of ^{14}C elimination (rapid and slow phases) for various target and nontarget tissues are summarized in Table 2; for the 0.15 mg/kg dose, the $t_{1/2}$ for the rapid phase ranged from 2.2 hr for blood to 4.2 hr for kidney while the $t_{1/2}$ for slow phase ranged from 11 hr for bone marrow to 29 hr for blood. All of the radioactivity in blood and tissues 1 hr after the 0.15 mg/kg dose appeared as benzene metabolites, indicating very efficient first-pass metabolism of benzene by the liver after oral absorption.

Accumulation in Zymbal Gland

The amount of benzene-derived material remaining in the Zymbal gland 24 hr after single gavage doses of 0.15, 1.5, 15, 150, and 500 mg/kg ^{14}C-benzene constituted less than 0.0001% of the administered dose, indicating that accumulation of ^{14}C does not occur in this gland. There was some evidence, based on tissue/blood ratios and extraction studies, that ^{14}C-benzene might be sequestered in mammary gland, bone marrow, and adipose tissue when animals were dosed with radiolabeled benzene at or above 15 mg/kg (Table 1). However, there was no indication that ^{14}C was selectively retained by the Zymbal gland in comparison to mammary gland and adipose tissue. Since it has been reported that fatty tissues (e.g., adipose, bone marrow) might function as a depot or sink for benzene (9,27), one experiment was carried out to specifically determine if ^{14}C-benzene residues accumulate in the Zymbal gland (a sebaceous gland) after repeated oral exposure. After 2 weeks of oral dosing at 500 mg/kg (single dose daily 5 days per week for two weeks), no apparent accumulation of ^{14}C was observed in the Zymbal gland compared to that after a single oral dose (data not shown).
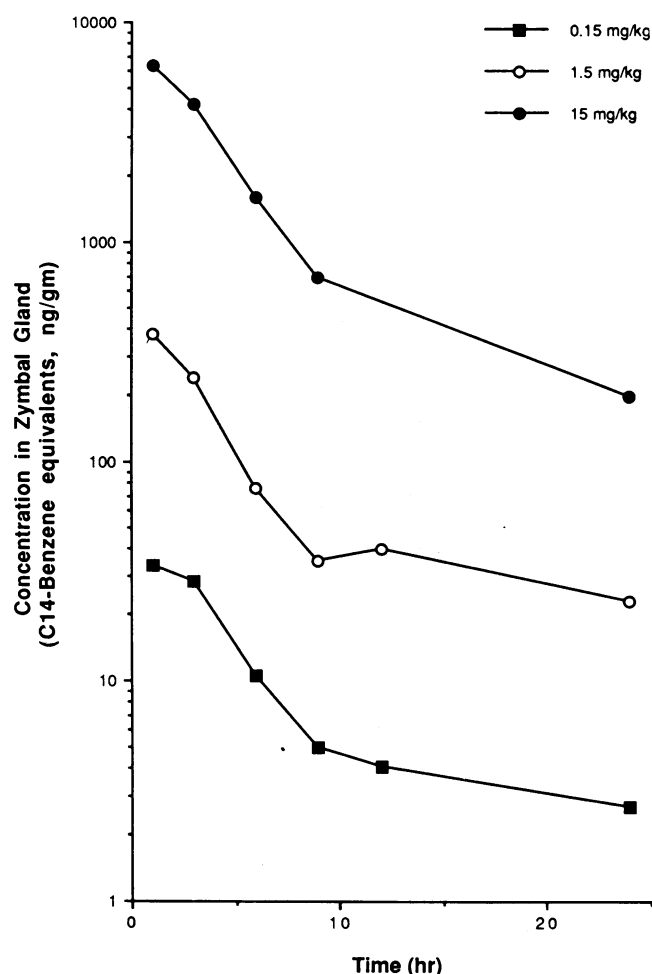

FIGURE 3. Comparison of ^{14}C levels in the Zymbal gland in rats given a single oral low dose of benzene of 0.15, 1.5 and 15 mg/kg. Points represent the means of three rats. For the 15 mg/kg dose group, tissues were not taken at the 12-hr time interval.

Table 2. Half-life of elimination of radioactivity from Zymbal gland and other tissues.[a]

Tissue	Dose, mg/kg	Rapid phase, $t_{1/2}$, hr	Slow phase, $t_{1/2}$, hr
Zymbal gland	0.15	2.8	18
Zymbal gland	1.5	2.4	21
Zymbal gland	15.0	2.5	8[b]
Blood	0.15	2.2	29
Blood	1.5	2.1	23
Blood	15.0	2.5	8[b]
Mammary gland	0.15	2.6	NC[c]
Nasal cavity	0.15	2.6	17
Oral cavity	0.15	2.9	23
Bone marrow	0.15	3.2	11
Liver	0.15	2.8	21
Kidney	0.15	4.2	14

[a]Half-life of elimination of radioactivity for the rapid and slow phases was estimated by visualizing the best line graphically on semilog plots and then best-fitting the data points for each phase using an exponential curve-fitting program on an HP 41CX calculator.
[b]Only two data points were available for determining half-life of the slow phase.
[c]Not calculable.

Metabolite Profile

The metabolites in Zymbal gland and other tissues were obtained by extraction, separated, and measured by HPLC and liquid scintillation spectrometry. Ethyl acetate extraction was used to isolate the unconjugated metabolites of benzene (e.g., phenol, hydroquinone, catechol, biphenol). HPLC separation of these free phenolic metabolites was achieved using an acetonitrile-ammonium acetate mobile phase (*26*). Separation of water-soluble metabolites of benzene (e.g., glucuronides, sulfates) was accomplished using an ion-pair HPLC gradient elution method (*22*). Radiochromatograms, such as those shown in Figure 4, illustrate the excellent baseline resolution of the water-soluble metabolites using the ion-pair HPLC procedure. The retention times for various standard metabolites performed under these two HPLC schemes are listed in footnote c of Table 3. The relative percentages of unconjugated and water-soluble metabolites found in Zymbal gland, oral and nasal cavity tissues, bone marrow, liver, kidney, and blood 1 hr after oral administration of 15 mg/kg ^{14}C-benzene are shown in Table 3.

Zymbal Gland. The major unconjugated metabolite identified in the Zymbal gland was hydroquinone ($\sim$ 30% of unconjugated metabolite fraction) but small amounts
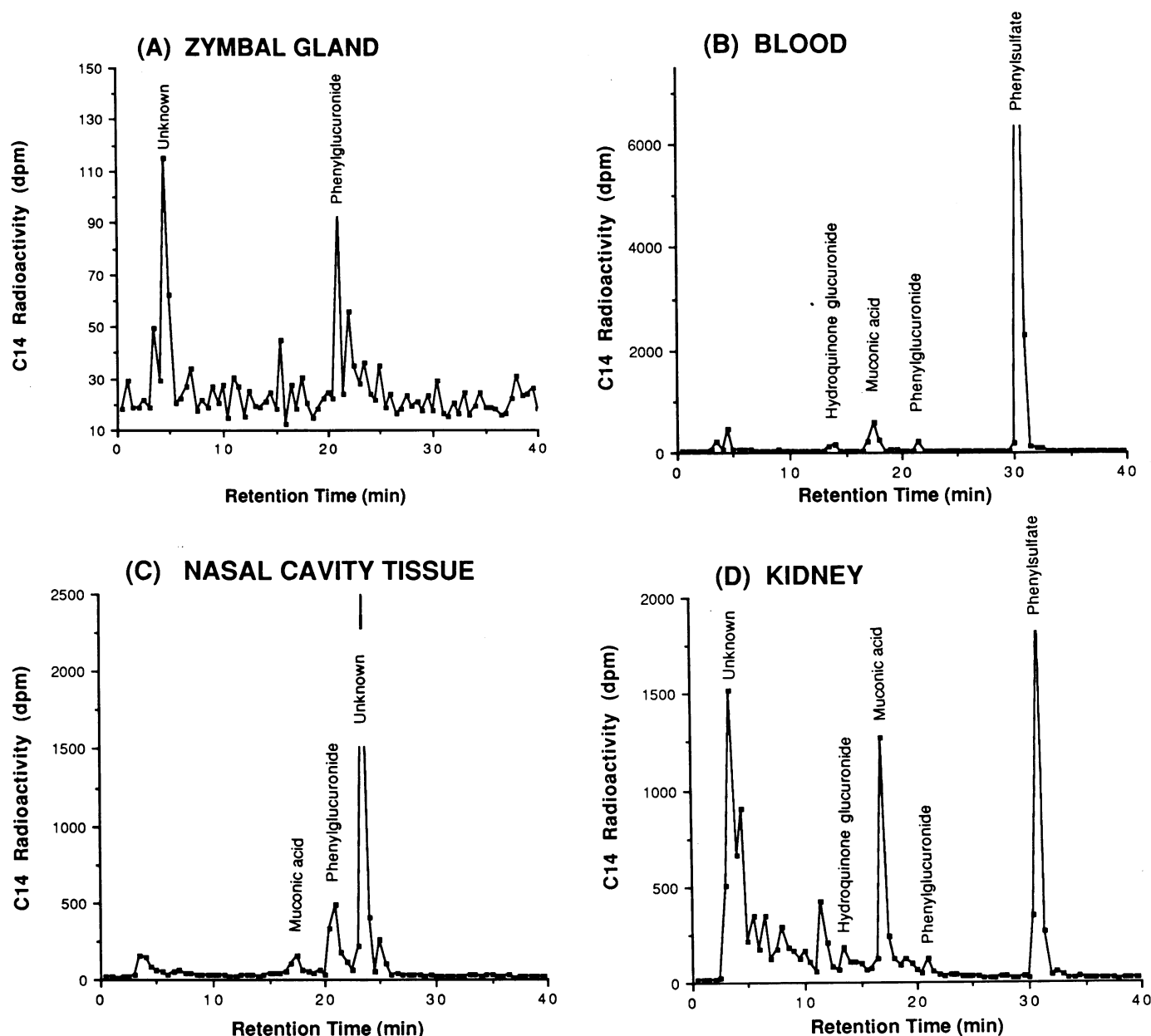

FIGURE 4. HPLC radioactivity profile of the water-soluble metabolite fraction isolated from various tissues and from blood 1 hr after female Sprague-Dawley rats were orally administered 15 mg/kg ^{14}C-benzene in olive oil: (*A*) Zymbal gland; (*B*) blood; (*C*) nasal cavity tissue; (*D*) kidney. Isolation procedures and ion-pair HPLC conditions are given in the materials and methods section. See Table 3 for relative percentages of each identified benzene metabolite and their HPLC retention times.

Table 3. Relative percentage of benzene metabolites in Zymbal gland and other tissues 1 hr after a 15 mg/kg ^{14}C-benzene oral dose.[a]

Metabolite	% Total radioactivity in ethyl acetate or aqueous fractions in tissue							
	Zymbal gland	Nasal cavity	Oral cavity	Bone marrow	Mammary gland	Blood	Liver	Kidney
Unconjugated metabolites in ethyl acetate fraction[b]								
Hydroquinone	30	11	53	—[d]	—[d]	64	89	65
Catechol	ND[c]	ND	ND	—	—	ND	ND	ND
Phenol	3	29	31	—	—	2	3	26
Unidentified	67 (1 major peak)	60 (1 major, 1 minor peak)	16	—	—	34	8 (2 peaks)	9 (2 peaks)
Water-soluble metabolites in aqueous fraction[b]								
Phenyl sulfate	ND	ND	62	66	—[d]	83	26	23
Phenyl glucuronide	35	18	3	ND	—	2	2	3
Muconic acid	ND	6	14	11	—	6	5	15
Hydroquinone glucuronide	ND	ND	4	2	—	2	8	6
Unidentified and other metabolites	65 (1 major, 2 minor peaks)	76 (major peak, 23.5 min, catechol glucuronide, 53%)	18 (4 minor peaks)	22 (4 minor peaks)	—	6 (2 minor peaks)	56 (major peak at 5 min, 27%)	53 (2 peaks at 3-5 min, 31%)

[a]See "Materials and Methods" for metabolite isolation procedure and HPLC conditions for separation of metabolites in the ethyl acetate fraction and in the aqueous fraction.

[b]HPLC retention times of unconjugated metabolites: 1,2,4-triol (3.0 min); hydroquinone (4.5 min); catechol (9.0 min); phenol (20 min). HPLC retention times (ion-pair) for water-soluble metabolites: hydroquinone glucuronide (14.0 min); triol glucuronide (major isomer, 16.0 min); muconic acid (17.5 min); phenyl glucuronide (21 min); catechol glucuronide (22.5 min); phenylsulfate (31 min).

[c]ND, not detected.

[d]Ethyl acetate extraction was carried out on bone marrow homogenate, but evaporation of solvent yielded little if any radioactive residue for HPLC analysis. Radioactivity lost most likely parent material. Similar findings occurred with mammary gland, radioactivity was not found in the evaporated ethyl acetate fraction or aqueous fraction.

of phenol (~ 3% of unconjugated fraction) were detected. However, about 67% of the radioactivity in the unconjugated fraction was associated with a polar, unknown peak having a retention time of less than 3 min. Efforts are underway to isolate sufficient quantities of this polar product from pooled Zymbal glands for mass spectrometric identification and structure elucidation. Free biphenolic metabolites were not detected in the Zymbal gland. Phenylglucuronide was identified as a water-soluble metabolite in the Zymbal gland based on retention time comparison with standards (Fig. 4A). Phenylsulfate and muconic acid were not detected as water-soluble metabolites in the Zymbal gland at 1 hr. The absence of phenylsulfate in the Zymbal gland was surprising since this sulfate conjugate was found as the principal water-soluble metabolite in blood (Fig. 4B), bone marrow, oral cavity, urine, and other tissues in our studies. About 65% of the radioactivity in the aqueous metabolite fraction of the Zymbal gland remained unidentified; most of the radioactivity was associated with a polar peak that elutes from the column at about 5 min (Fig. 4A, Table 3). The unknown polar peak does not appear to be prephenylmercapturic acid or a diconjugate based on relative retention times reported in the literature (22,28). However, the chromatographic nature of this polar product might be consistent with a structure such as a metabolite of muconic acid (e.g., shorter open-chain acid or alcohol metabolite) or a S-oxide metabolite of a mercapturic acid or premercapturic acid. Further characterization studies need to be carried out to evaluate these possibilities.

Blood. The unconjugated metabolites found in blood were hydroquinone and phenol, along with two unidentified polar products (Table 3). The HPLC profile of the water-soluble metabolites in blood 1 hr following a 15 mg/kg oral dose of ^{14}C-benzene is shown in Figure 4B. The major metabolite (31 min) coeluted with phenylsulfate and comprised 83% of the radioactivity in the aqueous fraction. In addition, muconic acid, phenylglucuronide, and hydroquinoneglucuronide were detected as minor metabolites (2 to 6%) in blood (Table 3).

Nasal Cavity Tissue. Unconjugated metabolites identified in the nasal cavity tissue included phenol (29% of unconjugated fraction) and hydroquinone (11%), while the major unknown metabolite (~ 60%) eluted as a polar peak on HPLC. The spectrum of water-soluble metabolites found in the nasal cavity tissue was different from that found in blood and other tissues in that phenylsulfate was not detected in the nasal cavity. Phenylglucuronide (18%) and muconic acid (6%) were identified as water-soluble metabolites in nasal cavity tissues, but a majority of the radioactivity in the aqueous fraction (76%) remained unidentified (Fig. 4C). The major radioactive HPLC peak eluted at 23.5 min and represented 53% of the aqueous fraction isolated from nasal cavity tissue.

Other Tissues. The metabolite profiles in various other tissues including bone marrow, oral cavity tissue, mammary gland, liver, and kidney have also been determined and are summarized in Table 3. The radioactivity found in the bone marrow 1 hr after a 15 mg/kg oral benzene dose was mainly parent and water-soluble metabolites (Table 3). Phenylsulfate, muconic acid, and hydroquinoneglucuronide were identified as the major water-

soluble metabolites in the bone marrow. In oral cavity tissue, hydroquinone and phenol were the principal unconjugated metabolites, while phenylsulfate, muconic acid, phenylglucuronide and hydroquinoneglucuronide were the primary water-soluble metabolites. Based on extraction results, the ^{14}C material present in mammary gland appeared to be the parent material. After attempted isolation of metabolites, practically no radioactivity was found in the aqueous fraction or the evaporated ethyl acetate fraction, indicating that the radioactivity initially associated with the ethyl acetate fraction was lost as ^{14}C-benzene during evaporation of ethyl acetate. Unconjugated metabolites detected in the kidney were hydroquinone and phenol. Phenylsulfate (23%) and muconic acid (15%) were the major water-soluble products while the glucuronide conjugates of phenol and hydroquinone were minor water-soluble metabolites (3 to 6%). The metabolite profile in the liver appears to be similar to that found in the kidney except that the relative percentages of muconic acid and unconjugated phenol were lower in the liver than in the kidney. A substantial portion of the water-soluble metabolites in Zymbal gland, liver, kidney, and nasal cavity tissue remained poorly characterized (Table 3); most of these products were polar in nature.

Discussion

Although solid tumor formation occurs in a number of tissues in rats following chronic oral benzene exposure (1-3), information has not been reported on the metabolic disposition and pharmacokinetics of benzene at these targets sites, which include Zymbal gland, oral and nasal cavity tissues, and mammary gland. Of particular interest is the Zymbal gland since this tissue is most susceptible to neoplastic changes induced by benzene. In this present study, we report the pharmacokinetics and metabolism of ^{14}C-benzene in Zymbal gland and other solid tumor target tissues in the Sprague-Dawley rat. Our results indicate that after oral administration, radiolabeled benzene is rapidly absorbed and distributed to the Zymbal gland, with peak levels reached within 1 hr of administration. Subsequent elimination of radioactivity occurs in a biphasic manner (rapid and slow phases). The slower elimination phase suggests a reduced rate of clearance of metabolites from the Zymbal gland, possibly because of covalent binding to tissue proteins. HPLC radiometric analysis demonstrated the presence of benzene metabolites in the Zymbal gland after treatment with ^{14}C-benzene. Free hydroquinone and phenol were detected as unconjugated metabolites, and phenylglucuronide as a water-soluble metabolite, on the basis of HPLC retention times. Other metabolites in Zymbal gland were polar in nature and have not been identified.

The metabolite profile found in the Zymbal gland differs from those found in blood and other tissues and is characterized by the absence of phenylsulfate. This finding is surprising since phenylsulfate is the predominant water-soluble metabolite of blood, liver, kidney, bone marrow, oral cavity tissue, and urine. These results are consistent with previous findings that sulfate conjugation may not occur readily in the Zymbal gland; Irving et al. (29) have reported that Zymbal gland lacks sulfotransferase activity toward N-hydroxy-2-acetylaminofluorene in the rat. Therefore, detoxification of phenolic xenobiotics in the Zymbal gland may be mainly proceeding by glucuronidation or possibly by other conjugative or metabolic pathways leading to polar products. The presence of phenylglucuronide and an unknown polar product as major water-soluble metabolites in the Zymbal gland of animals treated with benzene appears to support this hypothesis. The significant difference between the metabolite profile in blood versus that in the Zymbal gland suggests inherent metabolic capability in the Zymbal gland to biotransform benzene and/or its metabolites. If metabolites in the blood were transported to and sequestered by the Zymbal gland rather than being formed there, one would expect the two metabolic profiles to be more similar than distinctly different as experimentally observed. Pohl and Fouts (30) have demonstrated that Zymbal gland homogenates possess cytochrome P-450-dependent xenobiotic-metabolizing activity toward benzo[a]pyrene and 7-ethoxycoumarin. Studies in our laboratory using an in vitro tissue culture technique have demonstrated that the Zymbal gland is capable of metabolizing benzene, 2-acetylaminofluorene, and 7,12-dimethylbenzanthracene to reactive intermediates that interact covalently with DNA (31).

At this time, we cannot rule out the possibility that phenylsulfate might be taken up by the Zymbal gland (from surrounding blood supply); once in this organ, phenylsulfate could be rapidly hydrolyzed by sulfatase to yield phenol, which in turn, could be further oxidized and/or glucuronidated. The metabolic capacity of the Zymbal gland in regard to deconjugating or hydrolytic activities has not been well characterized (29,32). Relatively little is known on the distribution of sulfatases in the Zymbal gland (33). However, it is interesting to speculate that the presence of sulfatases in the Zymbal gland might provide a mechanism by which sulfate conjugates of phenolic benzene metabolites can be hydrolyzed to yield free phenolic metabolites, capable of undergoing further activation or inactivation processes. Work is underway to investigate this possibility.

Since the Zymbal gland is a specialized sebaceous gland in rodents (34), it is reasonable to expect that lipophilic chemicals like benzene would partition readily into the gland. However, results of these studies showed that accumulation of benzene does not occur in the Zymbal gland following single or multiple oral doses but that Zymbal gland metabolism produces a profile of metabolites, which is somewhat different from that of other organs and tissues examined. Perhaps this differential metabolism might contribute to the carcinogenic activity of benzene in the Zymbal gland and other target tissues. Continued metabolism and pharmacokinetic studies after single and repeated oral doses of benzene may provide further information for determining the likely reactive species and pathways involved in causing the formation of solid tumors in the rat.

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